








## RESEARCH ARTICLE

# Inbreeding intensifies sex- and age-dependent disease in a wild mammal

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**Abstract**

1. The mutation accumulation theory of senescence predicts that age-related deterioration of fitness can be exaggerated when inbreeding causes homozygosity for deleterious alleles. A vital component of fitness, in natural populations, is the incidence and progression of disease.
2. Evidence is growing for natural links between inbreeding and ageing; between inbreeding and disease; between sex and ageing; and between sex and disease. However, there is scant evidence, to date, for links among age, disease, inbreeding and sex in a single natural population.
3. Using ecological and epidemiological data from a long-term longitudinal field study, we show that in wild European badgers (*Meles meles*) exposed naturally to bovine tuberculosis (bTB), inbreeding (measured as multilocus homozygosity) intensifies a positive correlation between age and evidence of progressed infection (measured as an antibody response to bTB), but only among females. Male badgers suffer a steeper relationship between age and progressed infection than females, with no influence of inbred status. We found no link between inbreeding and the incidence of progressed infection during early life in either sex.
4. Our findings highlight an age-related increase in the impact of inbreeding on a fitness-relevant trait (disease state) among females. This relationship is consistent with the predictions of the mutation accumulation theory of senescence, but other mechanisms could also play a role. For example, late-life declines in condition, arising through mechanisms other than mutation accumulation might have increased the magnitude of inbreeding depression in late life.
5. Whichever mechanism causes the observed patterns, we have shown that inbreeding can influence age-dependent patterns of disease and, by extension, is likely to affect the magnitude and timing of the late-life declines in components of fitness that characterise senescence. Better understanding of sex-specific links between inbreeding, disease and ageing provides insights into population-level pathogen dynamics and could influence management strategies for wildlife reservoirs of zoonotic disease.

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## 1 | INTRODUCTION

Inbreeding depression describes a reduction in the fitness of offspring of related individuals (Charlesworth & Charlesworth, 1987; Darwin, 1876; Slate et al., 2004) and is explained by the expression of deleterious alleles that are made homozygous by breeding among related individuals. Inbreeding might harm components of fitness throughout an individual's life-course, or might, through a variety of mechanisms, become more harmful with increasing age. For example, the mutation accumulation theory of senescence (Medawar, 1952) predicts that natural selection on early-life fitness components should purge the deleterious effects of inbreeding in early life while late-acting effects remain, leading to an increase in the magnitude of inbreeding depression with advancing age (Charlesworth & Hughes, 1996; Williams, 1957; Wilson, Charmantier, & Hadfield, 2008). Mutation accumulation is not, however, the only mechanism that can yield changes in the magnitude of inbreeding depression with advancing age. The same changes could be caused by special cases of antagonistic pleiotropy (Charlesworth & Hughes, 1996; Williams, 1957) and by condition-dependent inbreeding depression when condition declines with age independent of accumulated late-acting mutations (Wilson et al., 2008).

Evidence is growing for natural links between inbreeding and ageing; between inbreeding and disease; between sex and ageing; and between sex and disease (all reviewed below). However, there is scant evidence, to date, for links among age, disease, inbreeding and sex in a single natural population. Understanding the causes and consequences of inbreeding depression has important applications for conservation (Hedrick & Garcia-Dorado, 2016; Keller & Waller, 2002) and for the epidemiology of wildlife and zoonotic disease (De Castro & Bolker, 2005). It is therefore important to consider interactions among age, inbreeding and host traits in our attempts to understand and manage disease in populations of wild animals.

Thanks to the existence of several exemplary demographic study systems, actuarial senescence (increasing risk of mortality with increasing age) and age-dependent declines in other fitness components have now been documented in natural populations (Beirne, Waring, McDonald, Delahay, & Young, 2016; Bérubé, Festa-Bianchet, & Jorgenson, 1999; Loison, Festa-Bianchet, Gaillard, Jorgenson, & Jullien, 1999; Nussey, Froy, Lemaître, Gaillard, & Austad, 2013), and genomewide homozygosity, caused by inbreeding, has been shown to exaggerate age-dependent trajectories of mortality and performance (Keller, Reid, & Arcese, 2008; Reynolds et al., 2007; Snoke & Promislow, 2003; Swindell & Bouzat, 2006; Wilson et al., 2007, 2008). In humans, inbreeding has been linked to the late onset of a range of diseases (Rudan et al., 2003). However, empirical data linking inbreeding depression to infection and disease are very limited in wild populations (Spielman, Brook, Briscoe, & Frankham, 2004; Townsend et al., 2009). Recent examples of inbreeding depression in immunological responses to pathogen exposure include song sparrows (*Melospiza melodia*) (Reid, Arcese, & Keller, 2003), American crows (*Corvus brachyrhynchos*) (Townsend, Clark, McGowan, Miller, & Buckles, 2010) and Tasmanian devils (*Sarcophilus harrisii*) (Siddle et al., 2007). Inbreeding has also been

linked to higher rates of parasitism in a range of species (Coltman, Pilkington, Smith, & Pemberton, 1999; Smallbone, van Oosterhout, & Cable, 2016; Whiteman, Matson, Bollmer, & Parker, 2006).

Sex differences in susceptibility to disease are well known in humans, domesticated and wild animals (Austad & Fischer, 2016; Bouman, Heineman, & Faas, 2005; Guerra-Silveira & Abad-Franch, 2013; Markle & Fish, 2014; Schuurs & Verheul, 1990; Zuk & McKean, 1996). Males are commonly more susceptible to disease in mammals (Klein, 2000; McDonald, Smith, McDonald, Delahay, & Hodgson, 2014); therefore, the strength of age dependence in disease incidence and severity might be expected to differ between the sexes. Divergent hypotheses regarding the link between sex- and age-dependent disease are perhaps equally valid a priori. If males are more susceptible to infection and disease-induced mortality, there should be greater selection pressure for early-life resistance or tolerance in this sex, at the expense of late-life effects, yielding a more intense age-dependent pattern in males relative to females. Alternatively, disease-induced mortality may be sufficiently intense among males that sublethal changes in disease incidence are simply undetectable in this sex.

Age-dependent patterns in infection and disease can have many proximate causes, including immunosenescence (age-related declines in immunological resistance to, or tolerance of, infection); behavioural changes with age (causing greater exposure to infection with increasing age); age-dependent sensitivity to diagnostic tests; and general declines in condition with age (causing greater incidence of disease, independent of the immune system). Surveys of disease incidence cannot readily tease apart the relative importance of these mechanisms leading to age dependence. Indeed, any population of hosts that suffer constant exposure to infection will display an increase in incidence and/or severity of disease with increasing age, simply due to the statistical process of cumulative exposure. Surveys can, however, detect heterogeneity in the slope of disease prevalence with increasing age, providing evidence for links between age dependence of disease incidence and host traits. To date, we know of only two examples that suggest a link between inbreeding and age-dependent disease, in wild animals. In song sparrows (Reid et al., 2003), disease resistance is determined by maternal effects in nestlings and by inbreeding depression among adults; and among wild Soay sheep (Coltman et al., 1999), inbreeding correlates with macroparasite loads in adults but not lambs.

Here, we study natural interactions between sex, age, inbreeding and infection status in European badgers (*Meles meles*) naturally exposed to *Mycobacterium bovis*, the pathogen responsible for bovine tuberculosis (bTB). There is evidence from a range of species that host genotype has an influence on the outcome of tuberculosis infection caused by members of the *Mycobacterium tuberculosis* (TB) complex (le Roex, van Helden, Koets, & Hoal, 2013). In humans, inbreeding depression has been associated with increased susceptibility to TB infection (Lyons, Frodsham, Zhang, Hill, & Amos, 2009). Similarly, inbred rabbits are more susceptible to TB infection under experimental infection conditions (Dorman et al., 2004), and inbred lines of mice segregate into resistant and susceptible phenotypes, with no intermediates (Briles, 2012). Inbreeding depression has been linked to increased susceptibility to *M. bovis* infection in African lions

(*Panthera leo*) (Trinkel, Cooper, Packer, & Slotow, 2011), red deer (Queirós, Vicente, Alves, de la Fuente, & Gortazar, 2016) and wild boar (Acevedo-Whitehouse et al., 2005).

In the UK and Ireland, the European badger is the principal wild-life reservoir of *M. bovis* and is involved in the transmission and persistence of bTB in cattle. The social structure, kin structure and dispersal patterns, typical of high-density badger populations, are known to influence mating patterns (Carpenter et al., 2005), increasing the likelihood of mating between relatives (Szulkin & Sheldon, 2008). Extra-group mating is commonplace (Dugdale, Macdonald, Pope, & Burke, 2007; Evans, Macdonald, & Cheeseman, 1989), with approximately half of all cubs fathered by males from other groups (Carpenter et al., 2005). It is possible that this behaviour evolved to militate against fitness costs associated with inbreeding (Annavi et al., 2014; Durrant & Hughes, 2005), perhaps helping to diversify the badger's major histocompatibility complex (MHC), a principal source of genetic resistance to disease (Sin, Dugdale, Newman, Macdonald, & Burke, 2012). Early work on population genetics suggested inbreeding rates among badgers in high-density populations were lower than those reported in other social mammals (Evans et al., 1989); however, more recent, pedigree-based findings reported that 5% of matings were among kin (Dugdale et al., 2007). The badger system is well suited to the study of links between age, sex and inbreeding, because age-related declines in immune cell telomere length and cytokine responses have been documented in both sexes (Beirne, Delahay, Hares, & Young, 2014; Beirne et al., 2016), and sex differences in components of immunity have also been reported (Beirne et al., 2016). Evidence of both reproductive (Dugdale, Pope, Newman, Macdonald, & Burke, 2011) and body mass senescence (Beirne, Delahay, & Young, 2015) have also been documented. Body mass senescence is also more intense among male badgers, and males also suffer significantly higher rates of disease progression and disease-induced mortality following infection with *M. bovis* (McDonald et al., 2014).

We investigate the relationship between inbreeding, age, sex and disease status using data from a long-term study of badgers naturally exposed to *M. bovis* infection. We predict that individuals with higher inbreeding coefficients will be more likely to express antibody responses to infection, indicative of progressed disease. We further predict, based on the mutation accumulation model of ageing (Medawar, 1952), that this link between inbreeding coefficients and progressed disease will intensify with age. We also propose that this link between inbreeding and age-dependent patterns of infection will differ between males and females, but it is difficult to predict which sex will suffer age-dependent progression more acutely, or in which sex it will be most easily detected.

## 2 | MATERIALS AND METHODS

### 2.1 | Badger sampling and TB diagnostic tests

All data used in these analyses were collected from the capture-mark-recapture study of a wild population of badgers at

Woodchester Park in Gloucestershire (Delahay et al., 2013). Since 1976, badgers have been trapped up to four times a year. Since the start of the study, a range of clinical samples (sputum, faeces, urine, swabs of bite wounds or abscesses) have been routinely taken for the detection of *M. bovis* by culture (Gallagher & Horwill, 1977). Between 1990 and 2005, blood samples were subjected to the Brock ELISA antibody test (Goodger et al., 1994) which was replaced with the improved Stat-Pak antibody test in 2006 (Chambers et al., 2008). A gamma-interferon assay for the cytokines associated with the cell-mediated response to *M. bovis* (Dalley et al., 2008) was also introduced in 2006 (Delahay et al., 2013).

We considered only those badgers of known age (i.e., first trapped as cubs) and for which we had a genotype. The badger mark-recapture database comprises a majority of individuals that never tested positive to *M. bovis* infection. For analyses, we included only those badgers whose lifetime set of diagnostic test results indicated they had been exposed to *M. bovis*. All exposed badgers contributed their entire set of capture data and diagnostic test results to the analysis. Although all the diagnostic tests have their limitations, the combination of test results provide biologically meaningful information on the incidence and subsequent progression of infection within individuals (Tomlinson, Chambers, Carter, et al., 2013; Tomlinson, Chambers, Wilson, McDonald, & Delahay, 2013). TB progression in badgers is assumed to be one way (i.e., infected individuals do not recover) (Gallagher, Monies, Gavier-Widen, & Rule, 1998; Graham et al., 2013). Outcomes of the different diagnostic tests reflect different stages of infection, from early (cell-mediated response, as measured by the gamma-interferon assay), to progressed (serological responses measured by Brock ELISA and Stat-Pak tests (Goodger et al., 1994; Chambers et al., 2008) to advanced ("infectious" individuals excrete *M. bovis* bacilli at single or multiple locations (Pritchard et al., 1986; Graham et al., 2013), as detected by culture). Limitations in the sensitivity of these tests have been explored in detail elsewhere (Buzdugan, Chambers, Delahay, & Drewe, 2016; Drewe, Tomlinson, Walker, & Delahay, 2010). However, all tests used are highly specific (gamma-interferon assay: 97% specific (Buzdugan et al., 2016; Hodgson & McDonald, 2018), Brock ELISA: 94%–98% (Clifton-Hadley, Sayers, & Stock, 1995; Goodger et al., 1994), Stat-Pak: 97% (Buzdugan et al., 2016) and culture: 100% (Drewe et al., 2010; Pritchard et al., 1986)). We are therefore confident that those individuals identified as infected in this study are not truly uninfected.

### 2.2 | Genotyping and measures of inbreeding

On first capture, a hair sample was taken from trapped badgers and stored in 80% ethanol prior to DNA extraction and genotyping (Carpenter et al., 2005). Genotyping involved the use of 22 microsatellite markers, each with 4–7 alleles. The MicroDrop Programme (Wang & Rosenberg, 2012) was used to impute missing microsatellite data. Deviations from Hardy-Weinberg equilibrium for each of the 22 microsatellite markers were tested on the imputed dataset using the hwtest function in "adegenet," and none were identified.

Homogeneity of variance among loci was confirmed ( $p = 0.78$ ) using Bartlett's test.

In the absence of a detailed pedigree, inbreeding was inferred using measures of multilocus heterozygosity (Jombart, 2008; Keller & Waller, 2002) using the MLH function in the `INBREEDR` package (Stoffeet al., 2016). An individual inbreeding coefficient (IIC) can also be estimated directly from microsatellite markers, where it is defined as the probability of an individual inheriting two identical alleles from a single ancestor. We checked the robustness of our MLH analyses using estimates of IIC, calculated using the R package `ADEGENET` (v 1.3-9.2) (Jombart, 2008). Multilocus heterozygosity, and IIC, was scaled to have zero mean and unit variance, prior to all analyses.

### 2.3 | Statistical modelling

All statistical analyses were conducted in R version 3.3.2 (R Core Development Team, 2016), using mixed-effects models using the package `LME4` (v1.0-5) (Bates, 2010). In all analyses, we regressed the antibody response (BrockELISA or Stat-Pak antibody test result) of each badger on each sampling occasion, as a binary response variable (antibody positive or negative), against the age (measured in quarter-years), homozygosity (measured as 1-MLH) and sex of the badger, and their interactions. As there is evidence that antibody test sensitivities increase with TB progression in badgers (Chambers et al., 2008; Goodger et al., 1994), the diagnosis "antibody positive" is here assumed to provide evidence for progressed infection, rather than evidence for successful defence against infection. We included the random effect of badger identity to account for the repeated measures of recaptures during a badger's life. We also accounted for variation in the prevalence of infection in the population among years by including year as a random effect. Finally, we considered the population structure among badgers by including the social group territory in which each badger was captured, as a third random effect. The key hypothesis was that any relationship between antibody response to exposure, and age, should be intensified by inbreeding, possibly with sex-related differences. This required testing of the homozygosity-by-age-by-sex interaction. We checked the robustness of this test by fitting the main effect of age as either a continuous or categorical variable (with age classified into quarter-year categories, or half-years when sample sizes became too small). We also checked the robustness of our result by replacing homozygosity with IIC in our regression models.

Links between homozygosity and fitness can be caused by direct effects, in which individual marker loci are responsible for fitness consequences, local effects, in which individual loci are linked to genes that affect fitness; and general effects, in which inbreeding depression is caused by genomewide homozygosity (David, 1998). We distinguished between local and general effects of inbreeding by regressing antibody response against age and the homozygosity at each marker locus, using separate models. We used the same random effects structure (badger, year, social group). Individual loci were judged important if the relationship between antibody response and homozygosity, or homozygosity interacting with age and

sex, remained significant following Bonferroni correction for multiple testing. Those individual marker loci, found to influence antibody responses significantly, were filtered from the dataset, and the MLH analysis was repeated using only the nonsignificant loci.

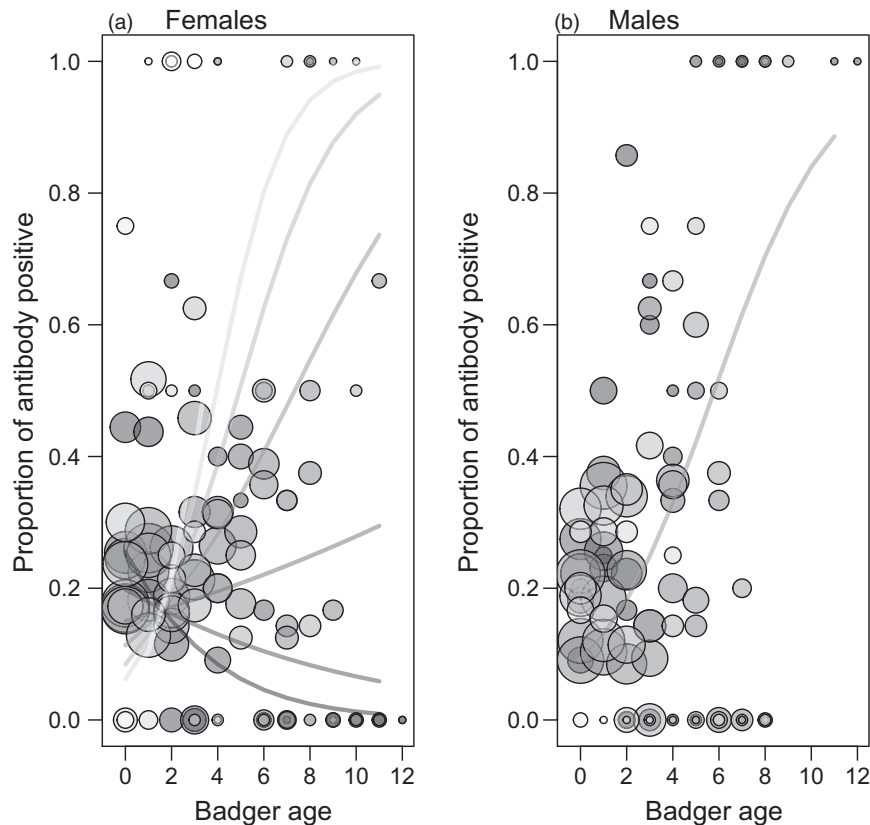
### 2.4 | Checking assumptions

A classic problem with tests for homozygosity-fitness correlations is the risk that effects are masked when variance in inbreeding is low. Identity disequilibrium is used as a proxy measure of variance in inbreeding; indeed, significant identity disequilibrium is a standard test to ensure that analyses of inbreeding effects have sufficient statistical power (Miller & Coltman, 2014). To test for the presence of identity disequilibrium (David, 1998), the  $g_2$  statistic and its standard error for the badger genotypes were calculated using the "`g2_microsats`" function within the R package `INBREEDR` [51]. Following the methodology in a similar study (Harrison, York, Cram, & Young, 2013), a randomisation approach was used to quantify the correlation between inbreeding estimates and heterozygosity (Balloux, Amos, & Coulson, 2004) and to calculate the heterozygosity-heterozygosity correlation (Balloux et al., 2004) between microsatellites. Weak but significant identity disequilibrium was detected in the genotype dataset of the whole Woodchester Park population ( $g_2 = 0.005$ ,  $SD = 0.0006$ ,  $p = 0.01$  based on 100 iterations). A significant heterozygosity-heterozygosity correlation was detected, consistent with the presence of identity disequilibrium ( $HHC = 0.07$ ,  $CI\ 0.03-0.11$ ). Therefore, the heterozygosity of the marker loci reflects heterozygosity at unlinked, functionally important loci; a key requirement for the general effect hypothesis of inbreeding depression (Slate et al., 2004). Heterozygosity and inbreeding estimates were significantly negatively correlated, based on 100 iterations ( $r = -0.08$  ( $CI: -0.10$  to  $-0.07$ ); this is in line with the expected correlation given the number of microsatellites employed and the population structure (Balloux et al., 2004) and suggests that the variation in heterozygosity among individuals is informative of their inbreeding level (Harrison et al., 2013).

We also checked for the Wahlund effect (Sinnock, 1975), in which links between homozygosity and fitness traits are not cause and effect, but are the outcome of population structure that clusters generally homozygous individuals in places that coincide with high or low trait values. This is important in our study system because high-density badger populations exhibit distinct social and spatial structure (Roper, 2010). We tested for heterogeneity in homozygosity among badger social groups and regressed group-level homozygosity against group-level disease incidence, using mixed-effects models with incidence as a binomial response and year as a random effect.

## 3 | RESULTS

The censored dataset comprised 3712 capture events, from 1990 to 2011, of 490 individual exposed badgers of known age. The



**FIGURE 1** The relationship between badger age and the likelihood of detection of antibody response to *Mycobacterium bovis* intensifies with increasing values of marker-wide homozygosity among females (a) but not males (b). Data are coloured by deviation from average homozygosity, measured in half-standard deviations from the mean, from dark grey (most-heterozygous individuals;  $-3$  standard deviations from the mean) to pale grey (most-homozygous individuals;  $+3$  standard deviations from the mean) and sized proportionally to the number of data points for each age-homozygosity combination. Fitted lines are from sex-specific generalised mixed models of antibody response against sex, age and homozygosity, including badger, year and social group as random effects. In the males-only model, there is no significant interaction between age and homozygosity, and no main effect of homozygosity. Colours of fitted lines follow the colour ramp of data points, from dark grey (most-heterozygous individuals) to pale grey (most-homozygous individuals)

likelihood of detecting an antibody response to bTB (here considered indicative of progressed infection; see above) increased with age. This result held whether age was modelled as a continuous ( $\chi^2_1 = 77.23$ ,  $p < 0.001$ ) or categorical (classified into quarter-year age intervals;  $\chi^2_{43} = 139.98$ ,  $p < 0.001$ ) main effect. This positive relationship between age and the probability of showing an antibody response became more positive with increasing homozygosity, but only among females (interaction between age, homozygosity and sex,  $\chi^2_1 = 16.43$ ,  $p < 0.001$ ; Figure 1; also robust to fitting age as a categorical main effect,  $\chi^2_1 = 19.41$ ,  $p < 0.001$ ). Overall, highly outbred female badgers showed a nonsignificant decline in immunological evidence for progressed infection with increasing age (test of relationship between disease progression and age in the 10% most outbred females;  $\chi^2_1 = 0.14$ ,  $p = 0.71$ , Figure 1a, red line). With increasing homozygosity, female badgers showed an increasingly positive relationship between age and evidence of progressed infection. Among males, we also observed increasing prevalence of progressed disease with increasing age, but the slope of this relationship did not depend on marker-wide homozygosity (Figure 1b). In both sexes,

there is no relationship between homozygosity and evidence of progressed disease in cubs (badgers aged less than 1;  $z$  test of slope using regression model with age as categorical variable classified into  $\leq 1$  year and  $> 1$  year;  $z_{\text{female} \leq 1} = -1.64$ ,  $p = 0.10$ ;  $z_{\text{male} \leq 1} = -0.62$ ,  $p = 0.54$ ). All results were robust to the replacement of multilocus homozygosity with IICs.

Five marker loci individually showed a highly significant link between homozygosity and antibody response (Table 1). One showed a significant main effect of homozygosity on antibody response. Two loci showed effects of homozygosity only as an interaction with age. Two loci revealed impacts of homozygosity only in interaction with age- and sex-effects. Locus m12 showed an influence of homozygosity as main effect, intensifying with age in both sexes, but especially among females. Following removal of these important loci, however, there remained a significant interaction between multilocus homozygosity and age, in their effect on antibody response ( $\chi^2_1 = 3.884$ ,  $p = 0.0488$ ). This residual effect of homozygosity was not sex-specific (interaction between age, sex and residual homozygosity,  $\chi^2_1 = 2.15$ ,  $p = 0.142$ ).

**TABLE 1** *p*-Values from a series of individual models to assess the strength of association between antibody responses to *Mycobacterium bovis* infection and homozygosity at each marker locus, in badgers from Woodchester Park (1990–2011)

Marker locus	Homozygosity <i>p</i> -value	Age: homozygosity <i>p</i> -value	Age: sex: homozygosity <i>p</i> -value
1bl	0.180	0.775	0.545
1bm	0.937	0.310	0.565
1bs	0.262	0.798	0.437
1bxl	0.045	<b><math>4.11 \times 10^{-4}</math></b>	0.410
1gl	0.098	0.285	0.067
1gm	0.205	0.005	0.807
1gs	0.691	0.143	0.012
1gxl	0.792	0.109	0.058
1ys	0.177	0.716	0.990
1yxl	0.733	0.017	0.533
2bs	0.388	0.306	<b><math>2.55 \times 10^{-7}</math></b>
2bxl	0.567	0.074	0.009
2gl	0.410	0.577	0.801
2gs	0.878	0.660	<b><math>1.6 \times 10^{-5}</math></b>
2gxl	0.053	0.086	0.107
2yl	0.364	0.026	0.286
2ys	0.869	<b><math>3.22 \times 10^{-5}</math></b>	0.670
m1	0.929	0.579	0.156
m10	0.009	0.046	0.047
m12	<b><math>5.12 \times 10^{-4}</math></b>	<b><math>4.41 \times 10^{-7}</math></b>	<b><math>9.70 \times 10^{-4}</math></b>
m14	0.060	0.109	0.908
m15	0.057	0.855	0.939

Note. In all cases, Wald's chi-squared tests were used to assess significance, based on mixed-effects models with binary response, locus homozygosity as fixed effect, and random effects of badger, year and social group. Significant results are bold if they satisfy the Bonferroni correction of  $\alpha^* = 0.05/22 = 0.0025$  to account for experiment-wide error rate.

Our test for the Wahlund effect (Sinnock, 1975) revealed that badger social groups varied significantly in levels of homozygosity ( $\chi^2_{37} = 124.34$ ,  $p < 0.001$ ), but with high levels of among-badger variation (Figure 2a). However, at the social group level there was no evidence of a link between mean inbreeding and the prevalence of positive antibody responses to *M. bovis* ( $\chi^2_1 = 0.10$ ,  $p = 0.747$ , Figure 2b). This suggests that the evidence for a link between inbreeding and age-dependent disease is not due to artefacts caused by population genetic structuring. We have controlled for any such effects using social group as a random effect in our mixed-effects regressions, above.

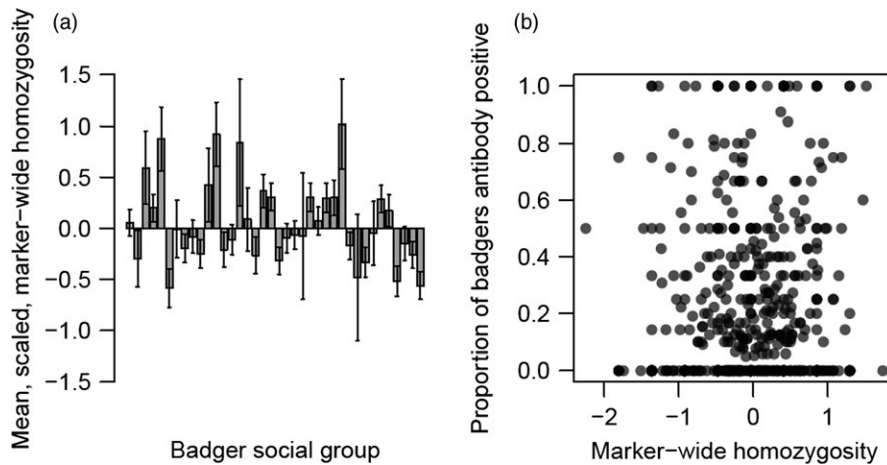
## 4 | DISCUSSION

We have demonstrated a link between multilocus homozygosity (indicative of inbreeding among badgers), age and the likelihood of detecting signs of progressed disease. This is evident mainly among female badgers. Males show an increase in disease incidence with increasing age, but with no link to inbreeding (except in our regression model that removed a subset of five important marker loci).

Similar links between inbreeding and age-dependent fitness components have been demonstrated for vital rates of reproduction (song sparrows; Keller et al., 2008) and contribution to population growth (red deer (Wilson et al., 2007)). Our findings provide a rare example of age-dependent inbreeding depression relating to disease in a wild mammal and are the first example, to our knowledge, of sex-dependent links between inbreeding and trajectories of disease with age. Our findings are also important because of the role of the badger in the epidemiology of bTB in cattle in the UK, Republic of Ireland and other parts of Europe.

We found evidence for local effects of homozygosity on the relationship between inbreeding, age and progressed disease, at a subset of genetic markers. These findings are consistent with the observation of genetic resistance to bovine TB in wild boar where only single-locus effects predicted TB progression, with several of the single loci being identified as mapping to regions of the genome with known immune function (Acevedo-Whitehouse et al., 2005). However, we also found residual evidence for general (and non-sex-specific) effects of multilocus homozygosity, following filtering of the individually important loci. This, coupled with significant identity disequilibrium in the badger population, is consistent with the





**FIGURE 2** Average levels of marker-wide homozygosity varied significantly among-badger groups (barplot a derived from mixed-effects model of homozygosity regressed against social group with year as random effect), but there was no significant relationship between the prevalence of antibody response to bTB infection and average homozygosity of the social group (scatterplot b)

“general effect” hypothesis for inbreeding depression, in which neutral marker loci are correlated with genomewide heterozygosity. We propose a combination of local and general impacts of inbreeding depression in this case: General and local effects represent two ends of a spectrum, and this population lies somewhere along the scale (Balloux et al., 2004).

In the absence of experimental challenge, and without knowledge of the timing of exposure to infection, we cannot tease apart the exposure, resistance and tolerance mechanisms that drive the age dependence of progressed disease detected here. The pattern could reflect increases with age in the cumulative probability of (a) a naive individual having been exposed to the disease and/or (b) an exposed individual having experienced disease progression. Alternatively, exposure, resistance and tolerance might themselves change with age, making them truly senescent drivers of age-dependent disease. This means that the influence of inbreeding could be on age-independent rates of exposure, resistance or tolerance, or on the pattern of senescence in these rates. Inbreeding could influence rates of exposure to disease via changes in behaviour or condition. It is also possible that age-related declines in components of immunity (as previously reported in this population [Beirne et al., 2014, 2016]) accelerate any underlying age dependence in the incidence of progressed infection. Here, we discuss these immunological and behavioural explanations, and their implications, in turn.

#### 4.1 | A possible role for immunosenescence

The observed age-related pattern of disease among females could arise in part from an effect of inbreeding on the rate of senescence in tolerance of infection with *M. bovis*. Both the antibody tests used in this study (Brock ELISA and Brock TB Stat-Pak) are more sensitive in individuals with progressed or disseminated disease (Chambers et al., 2008, 2009) which may pose the greatest risk of transmitting infection (Chambers et al., 2008). The pathogenesis of TB in badgers is complex, in that not all badgers exposed to *M. bovis* develop established disease, with a proportion mounting a successful immune response (Corner, Murphy, & Gormley, 2011). Badger macrophages fail to produce key defences against mycobacterial infection (Bilham

et al., 2017). In some cases, lesions develop and remain dormant, with badgers remaining in this latent phase throughout their lives (Corner et al., 2011; Gallagher & Clifton-Hadley, 2000). However, in a proportion of exposed badgers, the immune response is insufficient to contain the pathogen, resulting in active disease and excretion of bacteria (Corner et al., 2011; Gallagher & Clifton-Hadley, 2000). Inbreeding, and its effects on immune function in ageing badgers, might determine this transition from latency to progressed infection, and hence to infectiousness. Age-related declines in immune system performance have been demonstrated using ex vivo assays of components of immunity in badgers (Beirne et al., 2014, 2016). Individual homozygosity could therefore determine rates of attrition in immunocompetence in badgers, providing one potential explanation for the age-dependent trajectories of disease that we observe. Evidence of a role for senescence in disease resistance and/or tolerance can be gleaned from experimental pathogen challenges in individuals of different ages (e.g., Reid et al., 2003, 2007). A more tractable approach to investigating immunosenescence relevant to bTB infection in this study population will be to seek evidence of late-life increases in the age-specific probabilities of infection, disease progression and disease-related mortality (now a key focus of our work).

#### 4.2 | Behavioural change, age and inbreeding

The patterns we observed in this study are also consistent with a link between inbreeding and behaviours that cause increased exposure to *M. bovis* infection. It is also possible that inbreeding differentially increases the instantaneous risk of disease exposure among older badgers. For example, the late-life declines in body condition observed in badgers of both sexes (Beirne et al., 2015) could be exacerbated in more inbred individuals. Knock-on effects of such condition loss on social dominance could leave inbred, older badgers ostracised from their social groups, forcing them to use different resting sites or foraging opportunities, and perhaps bringing them into increased contact with environmental sources of infection or infectious badgers (or other host species). Social network studies have demonstrated that badgers with TB are

more prone to forming social links with members of other groups, making them of particular importance in disease transmission between groups (Weber, Bearhop, et al., 2013; Weber, Carter, et al., 2013). However, due to the unknown timing of infection, inferring any causal relationship between inbreeding, social behaviour and disease remains problematic: are socially connected badgers more likely to get infected, or does being infected change individual behaviour? The possibility that a similar phenomenon is observed among older and/or more inbred badgers has yet to be investigated.

### 4.3 | Sex differences in age dependence of inbreeding

We found evidence for links between inbreeding and age-dependent disease mainly in female badgers. This sex difference in intensity of inbreeding depression might be due to differences in longevity and the influence of bTB infection. Male badgers have an intrinsically higher mortality than females, regardless of their bTB infection status (Graham et al., 2013). Male badgers are known to experience faster progression of bTB once infected show weaker immune responses (Beirne et al., 2016; Tomlinson, Chambers, Carter, et al., 2013; Tomlinson, Chambers, Wilson, et al., 2013) and experience a higher rate of disease-induced mortality compared to females (Graham et al., 2013; McDonald et al., 2014). It is possible that investment in intrasexual competition diverts resource away from investment in immunity among males, lowering their tolerance of bTB infection (Beirne et al., 2015). In addition, infected male badgers may die off before they reach an advanced age, potentially explaining why no relationship between inbreeding and age was observed among males in the current study. Behavioural differences might also explain the sex specificity of disease trajectories (Graham et al., 2013; McDonald et al., 2014), because male badgers commonly range beyond social group boundaries and are more likely to engage in aggressive behaviours that might transmit infection via biting for example (Delahay et al., 2006; Macdonald, Harmsen, Johnson, & Newman, 2004).

The analyses presented here cannot determine whether links between inbreeding, disease and age are weaker among males or are instead not evident due to the selective mortality of infected and/or inbred males. We suggest that complex multistate models of age, infection status, sex and inbreeding (*sensu* (McDonald et al., 2016)) will be required to model simultaneously the processes of disease transmission, progression between disease states, survival and ageing, and how they depend on inbreeding in both sexes. Such multistate models would also help tease apart the processes of changes in exposure to and tolerance of infection with increasing age, and the cumulative and instantaneous effects of age on exposure and disease progression.

### 4.4 | Implications for management of bTB

The amount of infectious bacteria shed by an infectious badger is related to the extent of pathological progression (Nolan, 1991); hence, individuals with evidence of more progressed disease are likely to be more important in the onward transmission of infection

to susceptible individuals. From a management perspective, our evidence suggests that older, inbred females are more likely to exhibit progressed disease. However, their importance for disease spread will depend on whether they are also responsible for a greater share of infectious contacts (direct or indirect) with other hosts.

Our findings have relevance to the management of TB in badger and cattle populations. For example, variation in immunogenetic profiles among badgers may play an important role in *M. bovis* transmission and persistence within social groups and potentially scale up to population-level effects. If single loci are powerful predictors of TB progression, as suggested here and as found in wild boar (Amos & Acevedo-Whitehouse, 2009), then targeted management of susceptible genotypes might be an effective means of disease control. If inbreeding depression is indeed caused by genomewide homozygosity, then management intervention could target inbred individuals or groups, although this would inevitably require genetic surveys to identify inbred individuals. Targeted management could instead focus on older badgers. Alternatively, if behavioural mechanisms drive links between inbreeding and disease exposure or progression, then interventions could be targeted at places, or times, that are frequented by inbred badgers. An understanding of how the underlying genetic variation among badger populations impacts the severity and progression of disease would be valuable in modelling the spread of TB into new areas.

Additionally, management of TB in badgers through culling may alter the genetic structure of their populations, either by increasing levels of inbreeding through reducing density and an associated compensatory recruitment of cubs (McDonald et al., 2016), or by decreasing inbreeding as surviving individuals range more widely (Riordan, Delahay, Cheeseman, Johnson, & Macdonald, 2011), increasing mixing among remaining groups. Our results highlight the importance of considering the role that host genotype plays on disease outcomes, an area which until recently has been largely overlooked (Allen et al., 2010). Understanding how host genetics influence pathogen outcomes can help inform epidemiological models of disease spread (Hendricks et al., 2017) and, as recently demonstrated in the Tasmanian devil, can help to predict population-level responses to pathogens and inform conservation interventions such as translocations and reintroductions (Hendricks et al., 2017).

## 5 | CONCLUSIONS

Our findings strongly suggest that inbreeding intensifies an age-dependent increase in a fitness-related trait, the incidence of progressed disease, in a wild mammal population. This adds to growing evidence from natural populations in support of the mutation accumulation theory for the evolution of senescence (e.g., Wilson et al., 2007, 2008). That said, alternative explanations could also have played a role in generating this relationship. For example, condition-dependent inbreeding depression (Armbruster & Reed, 2005) could account for such a relationship independent of mutation accumulation, as (a) older badgers show reduced body condition (Beirne et al.,



2015) and (b) late-life declines in body condition could conceivably arise via mechanisms other than mutation accumulation (Wilson et al., 2008). Whatever the cause, these links among age, sex and inbreeding have implications for the transmission and prevalence of TB in this wildlife reservoir and in alternative host species. Our survey cannot tease apart the possible immunological, behavioural or condition-dependent drivers of disease progression among inbred badgers; hence, we recommend an experimental approach, using immunological challenge, in future studies. We also recommend: immunogenetic analysis of links between important marker loci and bTB resistance or tolerance; the study of links between inbreeding and behaviour (both social and spatial) among badgers of different ages; and multistate modelling that combines badger demography, bTB epidemiology and badger genetic profiles. Such studies could prove critically important to our understanding of this important wildlife reservoir of zoonotic disease and justify the deeper study of immunogenetics, ageing and inbreeding in other wildlife–disease systems.

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## AUTHORS' CONTRIBUTIONS

C.H.B., R.J.D. and D.H. designed the study and wrote the manuscript with input from A.J.Y., R.A.M. and F.A.P.S. C.H.B. and D.H. carried out all statistical analyses with input from A.R. Genotyping work was carried out by T.A.B. F.A.P.S. provided veterinary input on diagnostic test interpretation.

## DATA ACCESSIBILITY

Data from this study have been archived from Dryad Digital Repository: <https://doi.org/10.5061/dryad.sf34t61> (Benton et al., 2018). For those interested in collaborating in the use of data from the Woodchester Park population study, please e-mail [dez.delahay@apha.gsi.gov.uk](mailto:dez.delahay@apha.gsi.gov.uk).

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